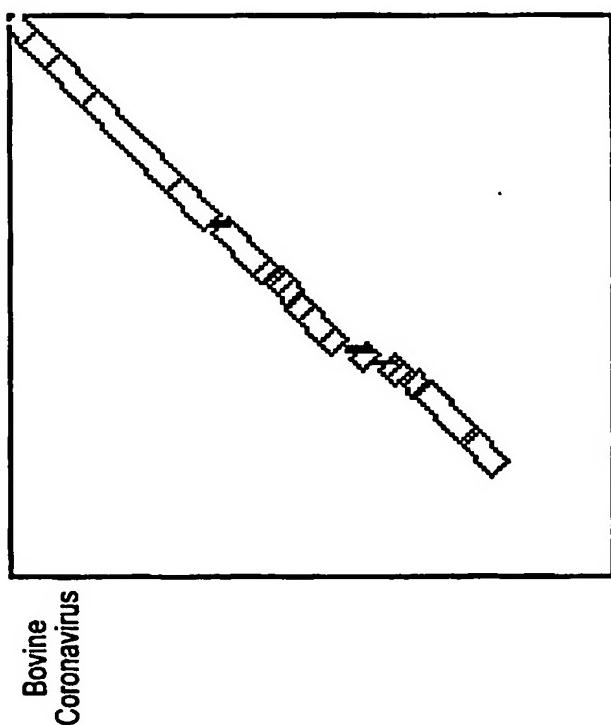
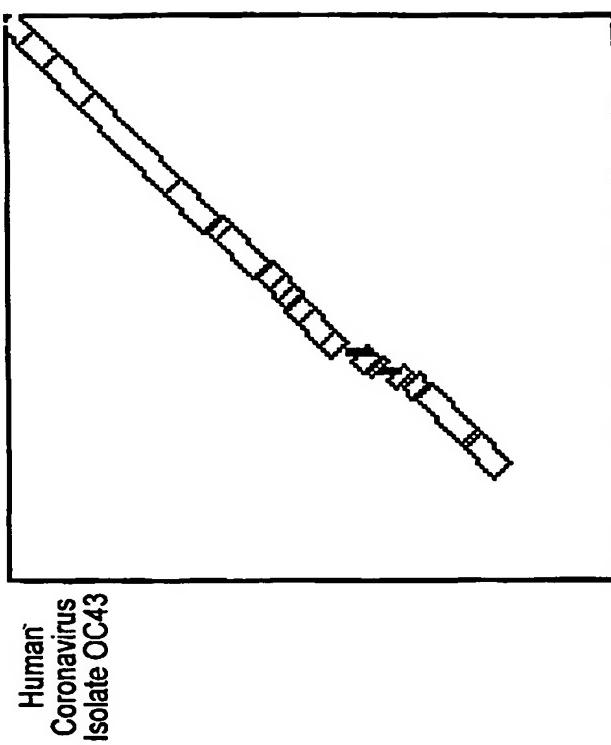


A.A. Sequence Comparison of Spike Protein between SARS Coronavirus with Bovine Coronavirus



Identities = 349/1122 (31%), Positives = 524/1122 (46%),  
Gaps = 148/1122 (13%)

A.A. Sequence Comparison of Spike Proteins between SARS Coronavirus with Human Coronavirus OC43



Identities = 349/1122 (30%), Positives = 524/1122 (45%),  
Gaps = 148/1122 (13%)

Figure 1

Figure 2

2/12

## Phylogenetic Analysis of Coronavirus N Protein

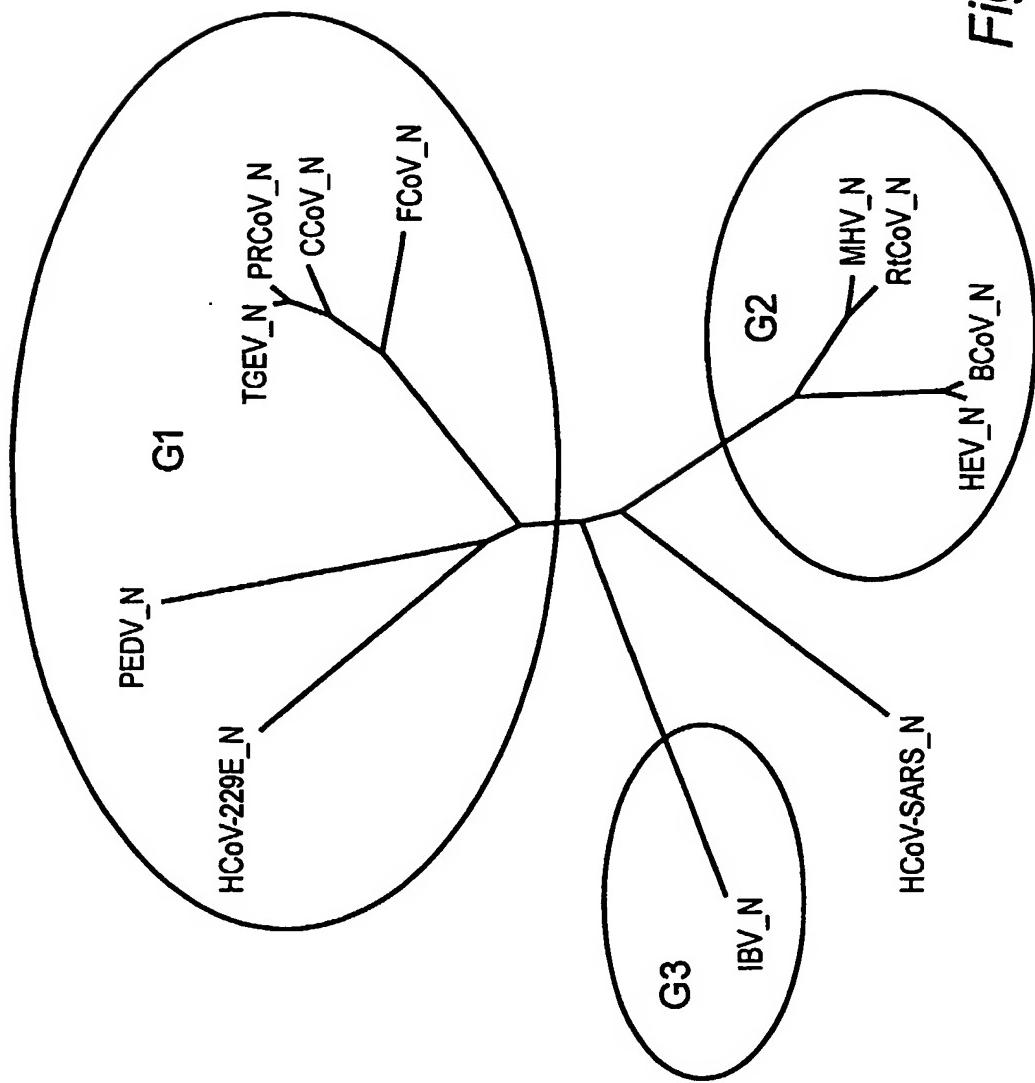


Figure 3

3/12

## Phylogenetic Analysis of Coronavirus S Protein

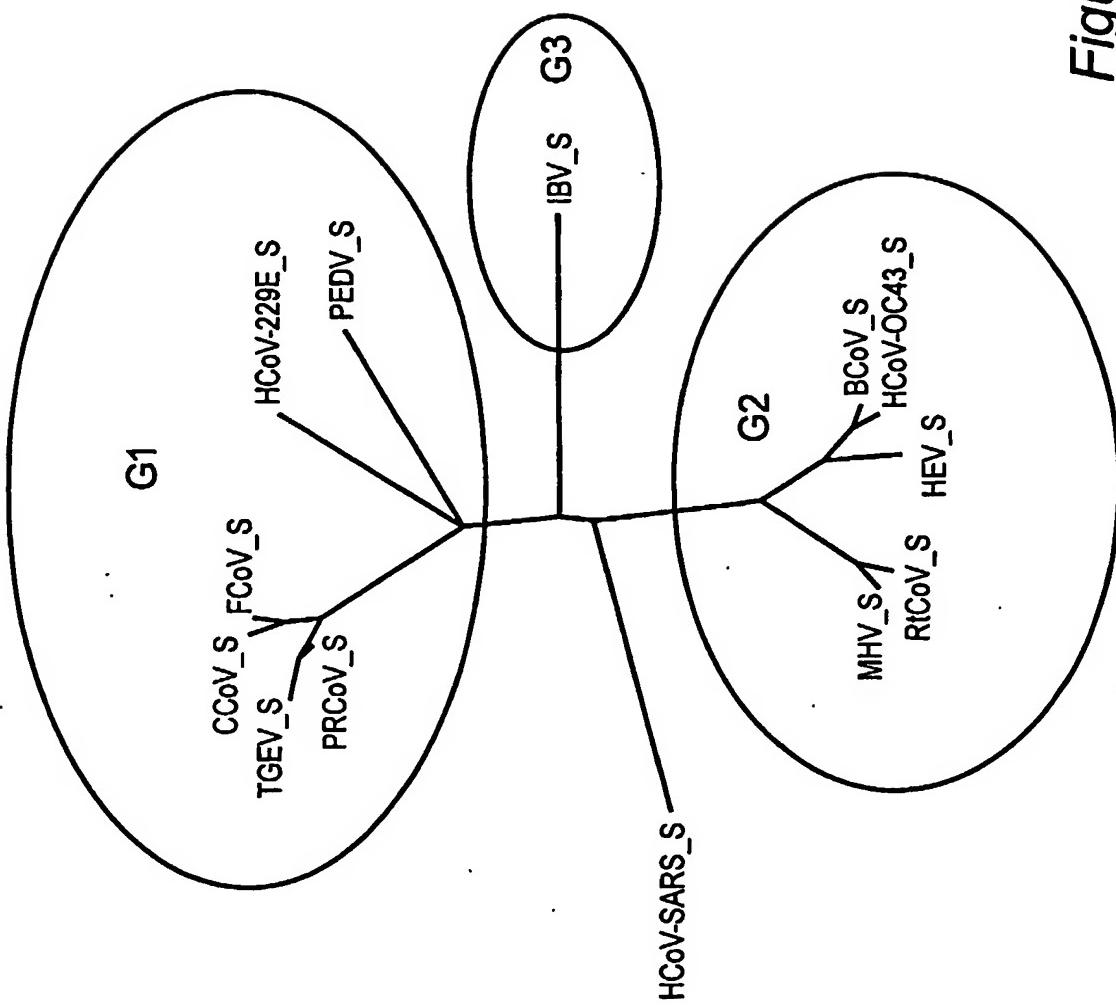
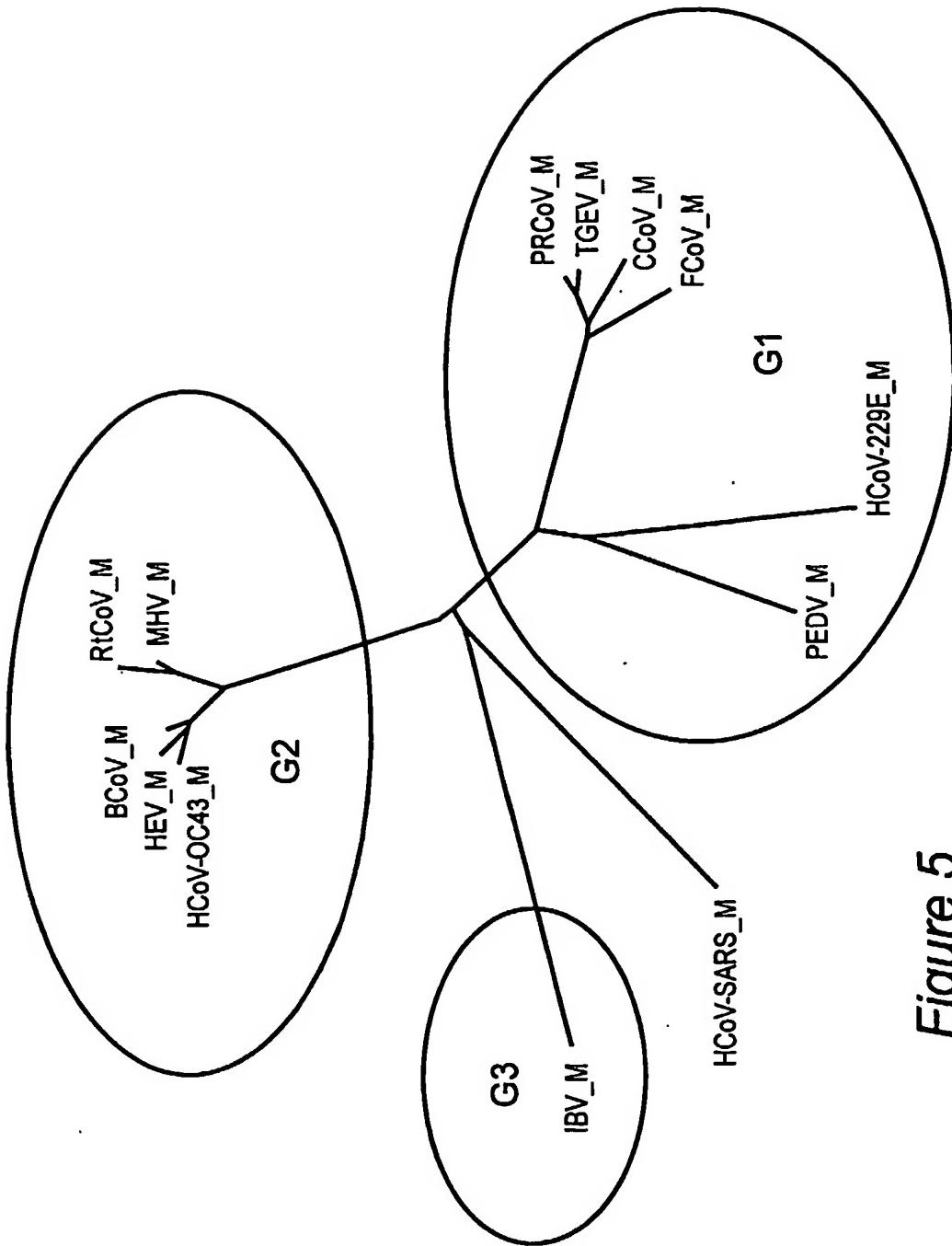
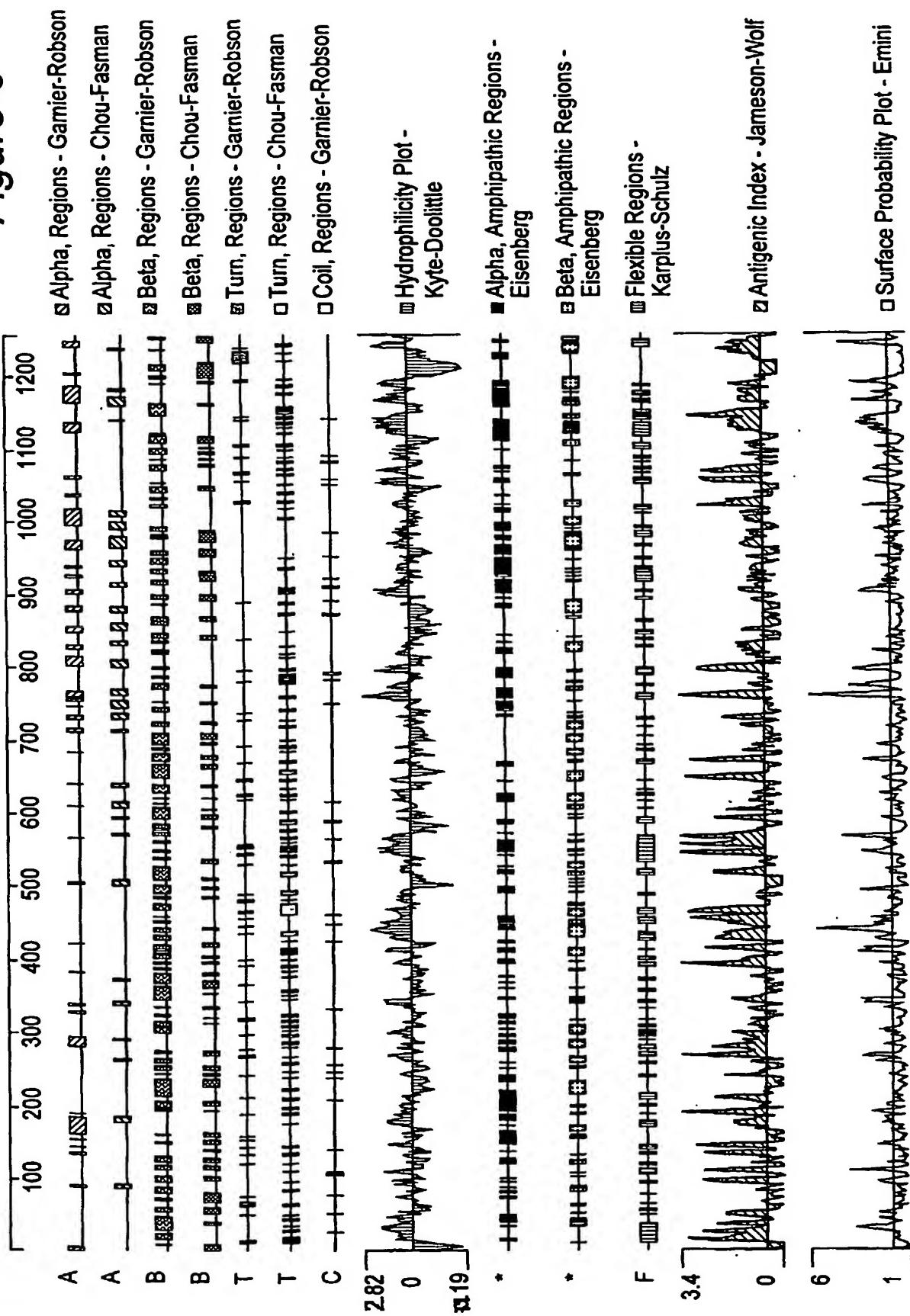


Figure 4

4/12

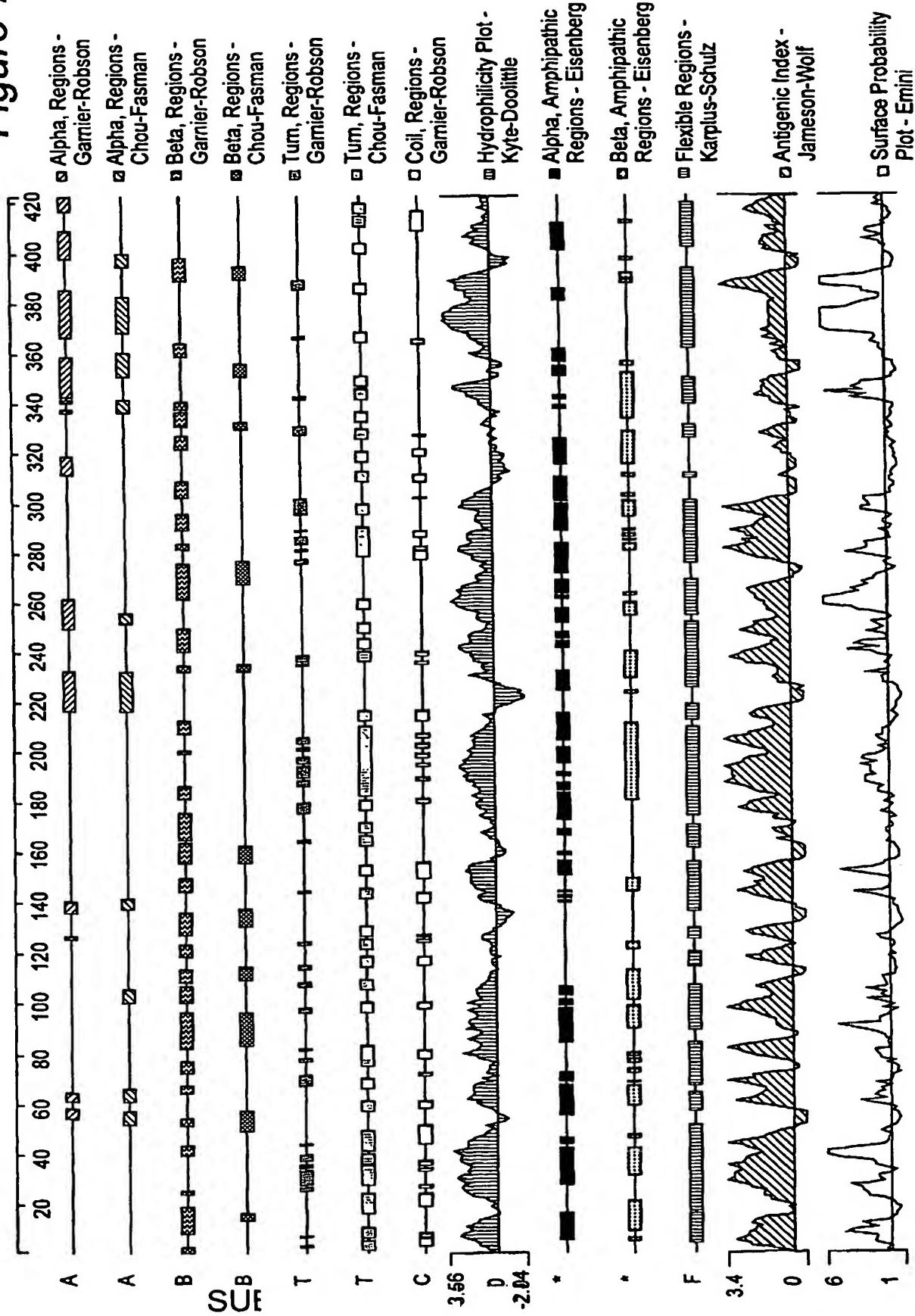
**Phylogenetic Analysis of Coronavirus M Protein****Figure 5**

5/12

**Figure 6****Protein Structure of SARS Coronavirus Spike Glycoprotein**

**Figure 7**

6/12

**Protein Structure of SARS Coronavirus NP Protein**

7/12

## SARS Spike Protein Peptides

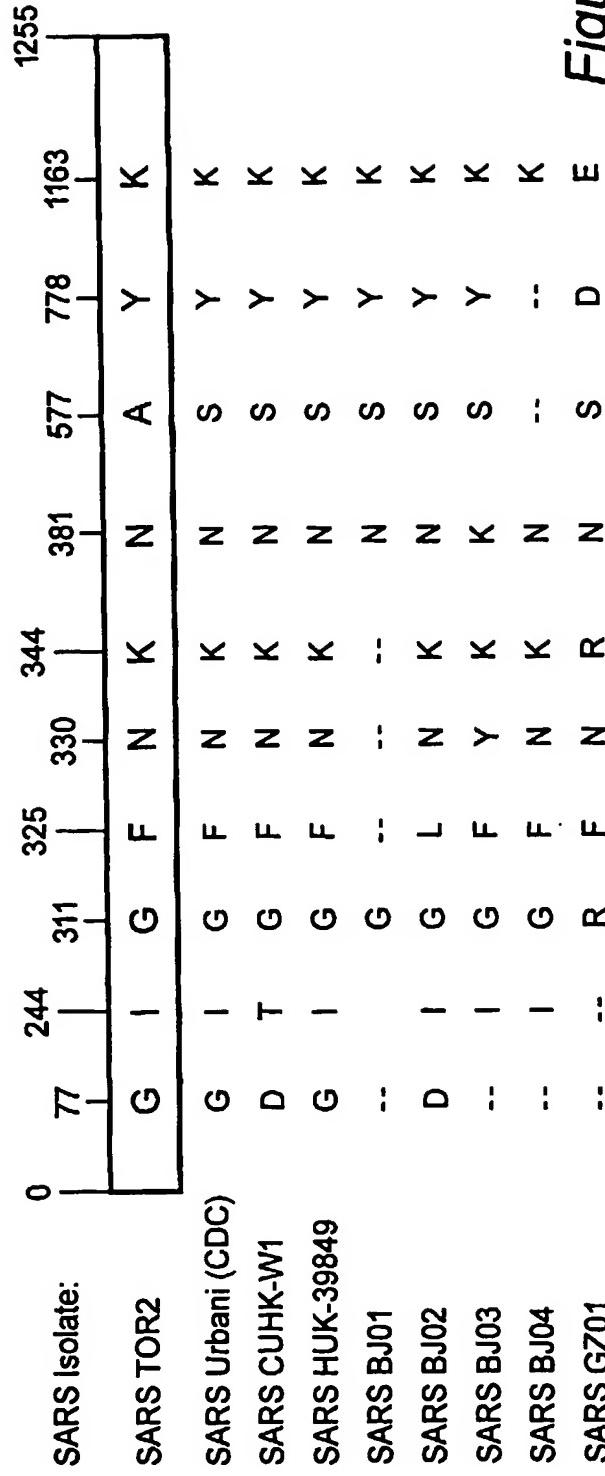
Name of peptide	Amino acid sequence	a.a position
DUHVI SA-S1	TTFDDVQAPNYTQHTSSSMRGGVYYPDEIFRSDT	20-51
DUHVI SA-S2	FKDGIVFAATEKSNVVRGMWVFGSTMNNKSQS	83-113
DUHVI SA-S3	NSTNVVIRACNFELCDNPFFAVSKPMGRQTH	119-149
DUHVI SA-S4	DVSEKSGNFKHLCREFVKNKDFGFLYYVKGYQPIDVVRDLPSG	171-213
DUHVI SA-S5	FSPAQDIWGTSAAAYFVGYLKPTTFMLKYDENGIT	238-273
DUHVI SA-S6	KYDENGITITDAVDCSONPLAELK	265-287
DUHVI SA-S7	FSPAQDIWGTSAAAYFVGYLKPTTFMLKYDENGIT	288-320
DUHVI SA-S8	FVVKGDDDVRRQIAPGQTGVIAADNYKLPODEM	386-417
DUHVI SA-S9	NTRNIDATSTGNINYKRYLRLRGKLRPFERDISN	424-457
DUHVI SA-S10	FSPDGKPCTPPALNCYWPLNDYGFYTITGIG	460-490
DUHVI SA-S11	PKLSTDLIKRNQCVNFNENGLTGTGVLTSSKRFO	513-546
DUHVI SA-S12	TPSSKRFQQFGRDVSDFTDSVRDPKTSE	539-569
DUHVI SA-S13	TNASSEAVLVLYQDVNCITDVSTAIHADQLTPAWRIYSTGN	588-626
DUHVI SA-S14	EHVDTSYECDIPIGAGICASYHTVSLLRSTSQKSI	640-674
DUHVI SA-S15	EHVDTSYECDIPIGAGICASYHTVSLLRSTSQKSI	753-782
DUHVI SA-S16	LKPTKRSFIELDLFNKVTLADAGFMKQYGECLGDINARDL	792-831
DUHVI SA-S17	NQKQIANQFNKAISQIQUESTTSTAAGKLQDVVNQNAQ	901-939
DUHVI SA-S18	SKRVDFCGKGYHLMSPFQAAPHGVVFLHVTYVPSQERNF	1019-1057
DUHVI SA-S19	EGKAYFPREGVFVFNGTSWFIITQRNFFSP	1066-1094
DUHVI SA-S20	DPLQPELDSFKEELDKYFKDNHTSPDVDLGDISG	1121-1153
DUHVI SA-S21	QKEIDRILNEVAKNLNESLIDLQELGKYEQY	1162-1191
DUHVI SA-S22	LTVLPPLITDDMIAAYTAALVSGTATAGWTFGAGAAALQIPF	841-882
DUHVI SA-S23	AMQMAYRFNGIGVTQNVLYENQKQIANQFNKAISQIESTL	843-921
DUHVI SA-S24	ELDSFKEELDKYFKXNHTSPDVDLGDISGINASVV	1127-1161
DUHVI SA-S25	NIQKEIDRILNEVAKNLNESLIDLQELGKYEQYIKWPW	1162-1197

Figure 8

8/12

**Figure 9****SARS NP Protein Peptides**

Name of peptide	Amino acid sequence	a.a position
DHV1 SA-N1	DSTDNNQNQNGGRNGARPKQRGPQLPNM	23-49
DHV1 SA-N2	GSRGGSQASSRSSRSRGNSRNSTPGSSRGNSPAR	176-210
DHV1 SA-N3	KVSGKGQQQQGTVTKSAAEASKKPROKRTATK	234-267
DHV1 SA-N4	GRRGPEEQTQGNFGDQDLIRQGTDYKH	276-301
DHV1 SA-N5	HIDAYKTFPPTEPKKDKKKKTIDEAQPLPQRQKKQ	357-369
DHV1 SA-N6	QKQKOPTVTLLPAADDMDDFSRQLQNSMSGASADSTQ	387-421

**Coronavirus Spike Protein Among Isolates**

-- = sequence not available

**Figure 10**

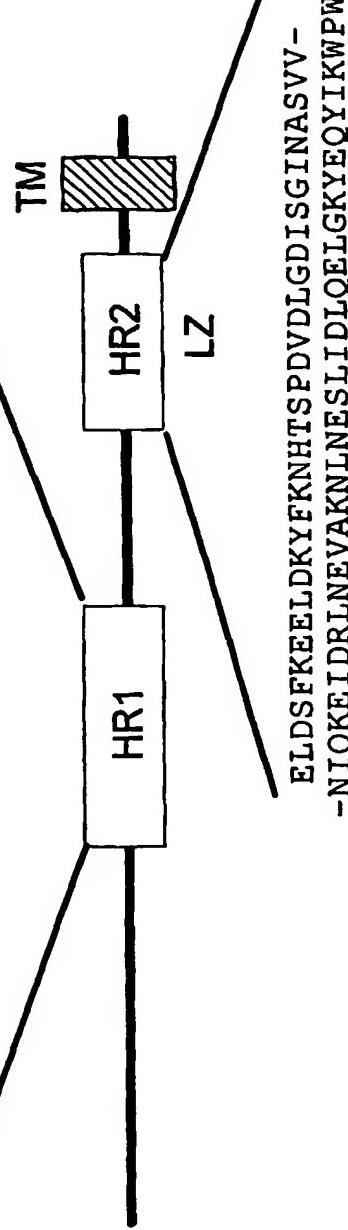
**Figure 11****Peptide Design Based on Predicated SARS Spike Protein Antigenic Epitopes**

Daniel C et al. J. Virol. 67:1185-1194, 1993; Routledge et al. J. Virol. 65:254-262, 1991; Talbot P.J. et al. J. Virol. 62:3032-3036, 1988.

10/12

### HR and LZ Domains in Coronavirus Spike Proteins

AMQMAYRFNGIGVTQNVLVYENQKQIANQFNKAISQIQESL-  
-LTVLPPPLTDDMIAAYTAALVSGTAGWTFGAGAAALQIPF



ELDSEKEEILDKYFKNHTSPDVDLGDISGINASVVV-  
-NIQKEIDRILNEVAKNLNESLIDLQELGKYEQYIKWPW

LZ

SARS TOR2

	<u>1125 P E L D S F K E E L D K Y F K N H T S P D V D L G - D I S G</u>
Hu coronavirus	1238 P N L P D F K E E L D Q W F K N Q T L V A P D L S L D Y - -
Bo coronavirus	1238 P N L H D F K E E L D Q W F K N Q T S V A P D L S L D Y - -
MHV	1105 P N L P D F K E E L D K W F K N Q T S I A P D L S L D F E K

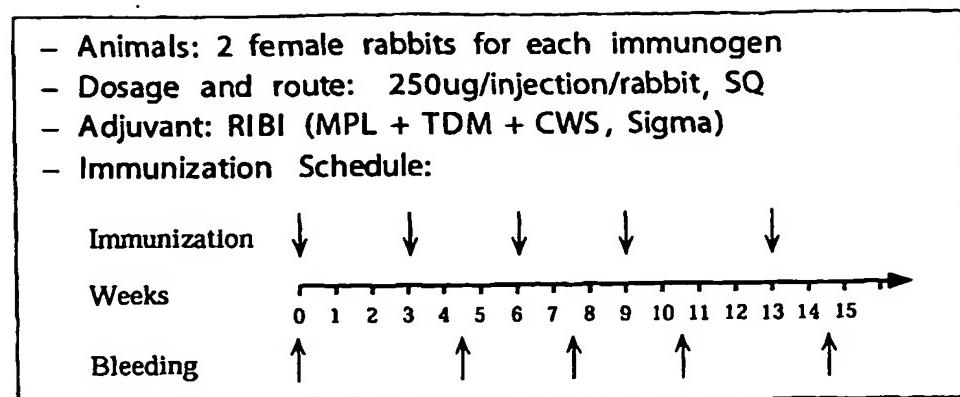
SARS TOR2

	<u>1154 I N A S V V N I Q K E I D R I N E V A K N I N E S L I D L Q E L G K Y E Q Y I K W P W</u>
Hu coronavirus	1266 I N V T F I D L Q D E M N R L Q E A I K V I L N Q S Y I N L K D I G T Y E Y Y V K W P W
Bo coronavirus	1266 I N V T F I D L Q D E M N R L Q E A I K V I L N Q S Y I N L K D I G T Y E Y Y V K W P W
MHV	1135 L N V T F I D L T Y E M N R I Q D A I K K I L N E S Y I N L K E V G T Y E M Y V K W P W

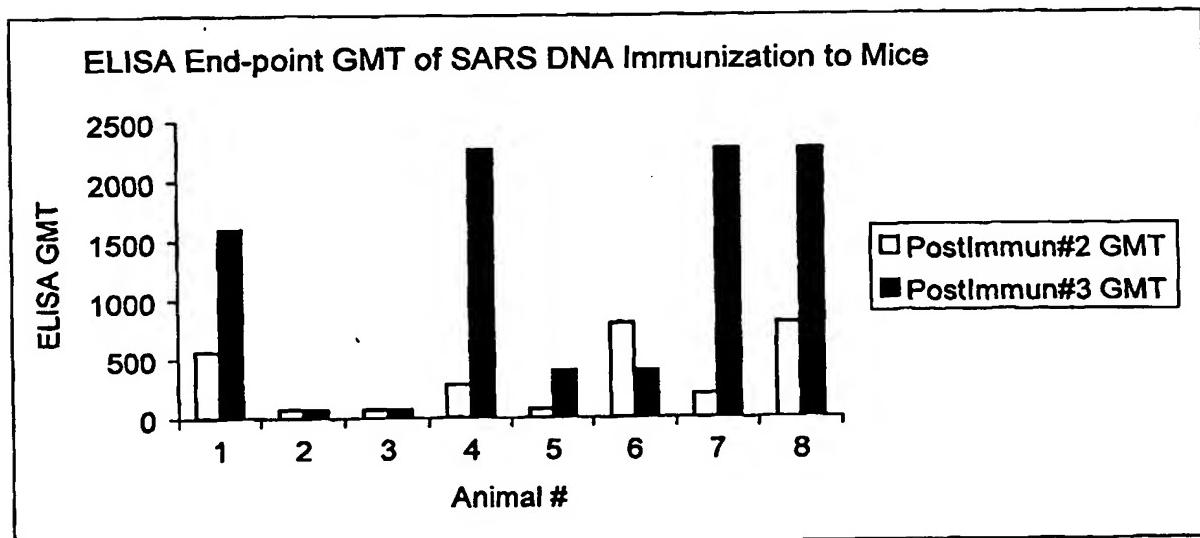
Luo, Z. and Weiss, S.R. In Coronavirus and Arteriviruses, ed by Enjuanes et al. Pp 17-22, 1998

Figure 12

11/12



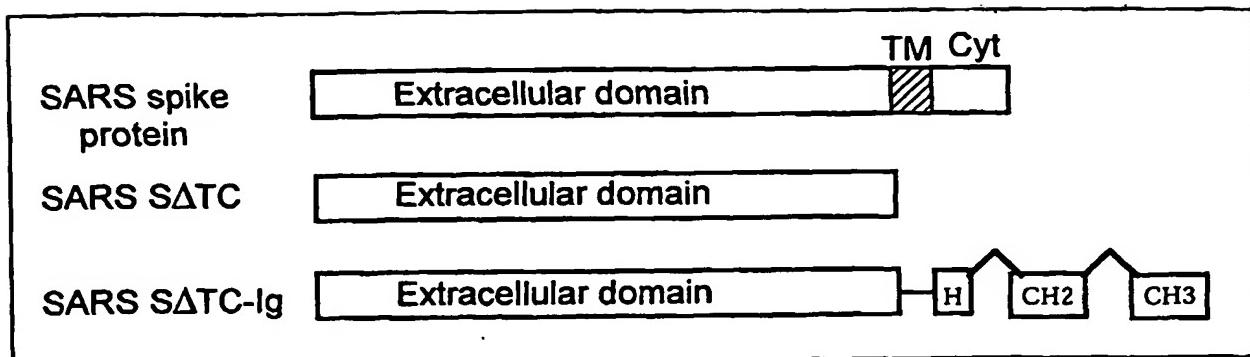
Immunization protocol of rabbits with SARS spike protein peptides

*Figure 13*

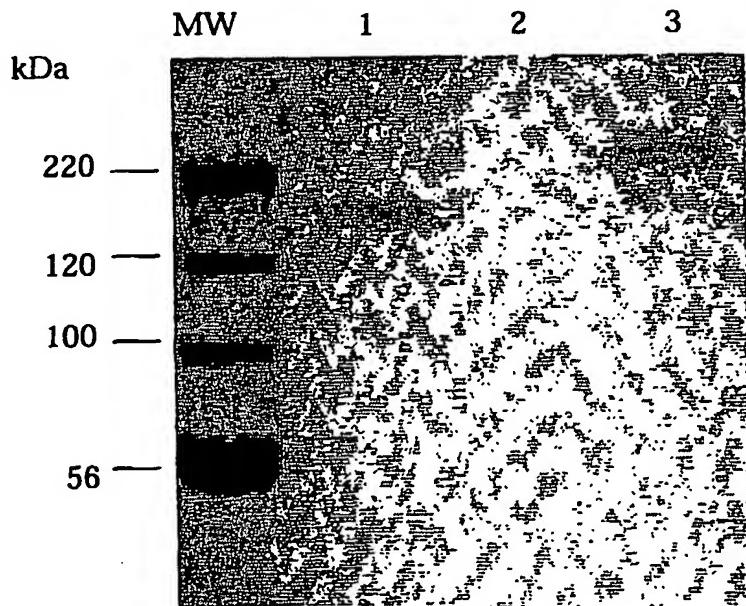
Induction of antibody reacted with recombinant SARS spike protein by immunization with plasmid DNAs express of SARS-spike protein or spike protein-Ig. Serum samples were collected 10 days after immunizations and assayed in ELISA. Show are the end-point ELISA titers against recombinant SARS spike proteins coated on 96-well plate (200ng/wll).

*Figure 16*

12/12



Schematic representation of SARS expression vectors

*Figure 14*

Western blot analysis of SARS spike proteins. Shown are purified SARS spike protein (lane 1), spike protein-Ig fusion protein (lane 3) and mock transfection supernatant control, which produced in 293 cells by transfection and purified by lectin column were analyzed in Western blot and detected by using immune sera of mouse immunized with DNA vaccine expressing SARS spike protein.

*Figure 15*